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ZISKA, S EXAMINER

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SARALYNN MANDEL
DIRECTOR OF INTELLECTUAL PROPERTY
CELL GENESYS, INC.
322 LAKESIDE DR.
FOSTER CITY, CA 94404

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This is a communication from the examiner in charge of your application.
COMMISSIONER OF PATENTS AND TRADEMARKS

☒ This application has been examined ☒ Responsive to communication filed on 6/11/94 ☐ This action is made final.

A shortened statutory period for response to this action is set to expire three (3) month(s), 0 (zero) days from the date of this letter.
Failure to respond within the period for response will cause the application to become abandoned. 35 U.S.C. 133

Part I THE FOLLOWING ATTACHMENT(S) ARE PART OF THIS ACTION:

1. ☒ Notice of References Cited by Examiner, PTO-892.
2. ☐ Notice of Draftsman's Patent Drawing Review, PTO-948.
3. ☒ Notice of Art Cited by Applicant, PTO-1449.
4. ☐ Notice of Informal Patent Application, PTO-152.
5. ☐ Information on How to Effect Drawing Changes, PTO-1474.
6. ☐ _____.

Part II SUMMARY OF ACTION

1. ☒ Claims 26-44, 46-70, 72-104 are pending in the application.

   Of the above, claims _____ are withdrawn from consideration.

2. ☒ Claims 1-25, 45, 71 have been cancelled.

3. ☐ Claims _____ are allowed.

4. ☒ Claims 26-44, 46-70, 72-104 are rejected.

5. ☐ Claims _____ are objected to.

6. ☐ Claims _____ are subject to restriction or election requirement.

7. ☐ This application has been filed with informal drawings under 37 C.F.R. 1.85 which are acceptable for examination purposes.

8. ☐ Formal drawings are required in response to this Office action.

9. ☐ The corrected or substitute drawings have been received on _____. Under 37 C.F.R. 1.84 these drawings are ☐ acceptable; ☐ not acceptable (see explanation or Notice of Draftsman's Patent Drawing Review, PTO-948).

10. ☐ The proposed additional or substitute sheet(s) of drawings, filed on _____. has (have) been ☐ approved by the examiner; ☐ disapproved by the examiner (see explanation).

11. ☐ The proposed drawing correction, filed _____, has been ☐ approved; ☐ disapproved (see explanation).

12. ☐ Acknowledgement is made of the claim for priority under 35 U.S.C. 119. The certified copy has ☐ been received ☐ not been received ☐ been filed in parent application, serial no. _____; filed on _____.

13. ☐ Since this application apppears to be in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under Ex parte Quayle, 1935 C.D. 11; 453 O.G. 213.

14. ☐ Other

EXAMINER'S ACTION

This application should be reviewed for errors.

The text of those sections of Title 35, U.S. Code not included in this action can be found in a prior Office action.

Claims 1-25, 28 -31, 45, 67, 68, 71, 72, 76, 83, 89, 90, 93, 99 and 101 have been cancelled; claims 26, 27, 32-44, 46-66, 69, 70, 73-75,77-82, 84-88, 91, 92, 94-98, 100, 102-104 are active and examined in this Office Action.

The declaration of Dr. Liskay is acknowledged, has been considered and is addressed, below.

The request to Make Special filed December 29, 1994, is acknowledged and this Office Action is responsive thereto.

It is noted for the record that the amendment to claim 100 was not entered due to inconsistencies between claim 100 as amended in amendment B and amendment C. Therefore, claim 11 stands as in amendment B.

The rejection of claims 26, 27, 32-44, 46-66, 69, 70, 73-75,77-82, 84-88, 91, 92, 94-98, 100, 102-104 under 35 U.S.C. 112, first paragraph, is as follows: the rejection of claim 27 and claims dependent therefrom, regarding the integration of the amplifiable gene within the endogenous gene, is maintained since the amendments to the claims do not overcome the rejection and in fact necessitate a new ground of rejection under 35 U.S.C. 112, second paragraph. The rejection of claim 67, for reasons of record, is withdrawn in view of the cancellation of the claim.

Applicants' amendments to the claims have necessitated the new grounds of rejection and this Office Action will be made Final.

The rejection of claims 26, 27, 32-44, 46-66, 69, 70, 73-75,77-82, 84-88, 91, 92, 94-98, 100, 102-104 under 35 U.S.C. 112, second paragraph, regarding the phrase "modified endogenous gene" is withdrawn in view of the amendments to the claims. However, as previously stated, there is no literal support in the parent ('069) specification for regulatory elements or sequences wherein the element or sequence represents a promoter and the first paragraph rejection is maintained.

Applicants' arguments, filed February 7, 1995, have been considered but not found to be persuasive. Applicants are not entitled to the filing date of the parent application 07/432,069, regarding the replacement of endogenous promoters with heterologous promoters using homologous recombination. Contrary to Applicant's assertions, neither the instant specification nor the parent specification provide literal support for the invention as claimed for reasons set forth in the previous Office Action and reexplained here. Applicants have disclosed targeting of the EPO locus and transfer of the recombined locus to the secondary host cell but have failed to disclose targeting and homologous recombination using nucleotide regulatory elements other than enhancers. Applicants have been asked to specifically point out support in the specification for the claims as amended

and they have done so in the instant amendment. Applicants have stated that the '390 parent application exemplifies using the CMV promoter/enhancer to activate expression of EPO at page 3, lines 12-17, page 7, lines 1-35 and p 15, lines 1- p. 19, lines 20. However, contrary to such arguments, page 3, lines 12-17 disclose integration of "an amplifiable gene and other regulatory sequences" in proximity to a gene of interest. Page 7, lines 1-35, provide for insertion of "a transcriptional initiation region different from the wild type initiation region", introduction of signal leader sequence (lines 16-18); alternatively, changing the 3' polyadenylation site; "Therefore, by homologous recombination, one can provide for maintaining the integrity of the target gene, so as to express the wild-type protein under the transcriptional regulation of the wild-type promoter or one may provide for a change in transcriptional regulation, processing or sequence of target gene. In some instances, one may wish to introduce an enhancer in relation to the transcriptional initiation region, which can be provided by, for example, integration of the amplifiable gene associated with the enhancer in a region upstream from the transcriptional initiation regulatory region or in an intron or even downstream from the target gene". Therefore, although the instant application discloses use of the CMV promoter/enhancer, the claims are not entitled to the filing date

of the parent application with regard to the use of promoters per se.

Applicants have argued that the '069 parent application specifically refers to "regulatory sequences" and that the '069 application then devotes at least two pages of the specification to a description of the regulatory sequences that can be targeted by homologous recombination and point with particularity to application '069, page 6, line 25 to page 7, line 21. However, "regulatory sequences" encompass many different elements and "regulatory sequence" per se is not analogous to, or identical with, "promoter". "Regulatory sequence" has no one meaning and contrary to Applicants' arguments, the '069 specification does not disclose use of promoters per se in homologous recombination in the practice of the invention. Contrary to Applicant's arguments, the '069 specification is confined to enhancers, and does not include promoters. Further, the specification does include the targeting of transcriptional initiation regions; however, it was well known to one of ordinary skill at the time the claimed invention was made that the "transcriptional initiation regions" were not synonymous with "promoters". The declaration of Dr.Liskay is acknowledged but fails to be persuasive on this issue. The evidence presented by Applicants regarding the definition of "promoter" is not acceptable since the publication date of the dictionary reference is too old to be

of value. One of ordinary skill in 1989 would not have considered the definition "promoter" as stated in by Old to be "state of the art" or commensurate with the skill of one of ordinary skill in the art. Indeed, one of ordinary skill in the art at the time the claimed invention was made would not have found the definition of promoter set forth by Old to be still meaningful. Applicants have argued that the '069 specification includes the targeting of transcriptional initiation regions, which they equate to be "promoters different from the wild type transcription regulatory sequences of the target gene" and cite the following passage as support:

> "Alternatively one could provide for insertion of a transcriptional initiation region different from the wild-type initiation region between the wild-type initiation region and the structural gene"
>
> and
>
> "Therefore, by homologous recombination, one can provide for maintaining the integrity of the target gene, so as to express the wild-type protein under the control of the transcriptional regulation of the wild-type promoter or one may provide for a change in transcriptional regulation, processing or sequence of the target gene".

Note that the phrase "so as to express the wild-type protein under the control of the transcriptional regulation of the wild-type promoter" clearly says that the wild-type protein is expressed under the control of its own (wild-type) promoter and that therefore, the promoter linked to its natural gene was transferred into the target site, not that the promoter alone was

transferred in. This then is the basis for the lack of support for the inclusion of heterologous promoters in the practice of the invention. The specification states that any promoter switched in by homologous recombination must be accompanied by its natural gene and not, as applicants have argued, any heterologous promoter alone.

Applicants have argued that "surely the examiner must appreciate that targeting of promoters is intended by the description of the '069 grandparent application". However, the examiner does not since the specification fails to clearly state such. Further, it is clear from the state of the art as well as the cited art, that at the time the claimed invention was made the ordinary practitioner clearly knew the difference between "enhancers", "promoters", "transcription initiation regions" and "transcription start sites". Support for the Examiner's position was established by the submitted references and can be further supported by the following:

Muller, in 1988, only 1 year prior to the filing date of the parent (432,069) application, was able to clearly set forth different elements of proximal and remote transcriptional control. See figure 1, page 486, wherein 4 different situations were at that time known in the art: A, wherein the enhancer/promoter were the same since they overlap; B, wherein the enhancer and promoter are separated by several thousand base

pairs; C, wherein the enhancer and promoter are separate and the enhancer exists within an intron; and D, wherein the promoter and enhancer are separate and the enhancer is in the 3' region. Therefore, Muller provides support for the Examiner's position that at the time of the filing of the '069 parent application to which applicants are claiming priority, the specification does not disclose or contemplate the use of "promoters" since applicants would clearly have known about "promoters" and the fact that they are distinct from other regulatory elements although in one case the promoter overlaps with the enhancer.

Applicants have argued that the Liskay declaration concludes that "taking into account the knowledge and understanding of one skilled in the art of molecular biology would have had in 1989, the teachings of the '069 application are not limited to the targeted integration of enhancers, but include the targeted integration of any and all promoter elements different from the wild-type or native promoter that controls expression of the target gene". However, it must be pointed out that there is a vast difference between actual inclusion of specific material in a patent application and the statements by a declarant of what one of ordinary skill in the art would have "understood" to be there. In view of the fact that "promoters" as a regulatory entity were old and well known in the art, as evidenced above, that "promoters" were clearly distinguished from enhancers as

well as "transcription initiation regions" Applicants' arguments, as well as the Liskay declaration are not persuasive on this issue.

Applicants have argued that the text itself refers to changing the transcriptional regulation region so that it is different from the wild-type promoter. However, this statement is erroneous; the specification refers to:

"so as to express the wild-type protein under the control of the transcriptional regulation of the wild-type promoter or one may provide for a change in transcriptional regulation"

which clearly states the use of the wild-type promoter with the wild type protein and does not include switching in a promoter which is different from the promoter naturally occurring with the gene of interest. Applicants use of Old and Primrose as the source of the definition of "promoter" is inappropriate since one of ordinary skill at the time the claimed invention was made would not have found that definition to be at the level of one of ordinary skill. One of ordinary skill would have accepted the definition of "promoter" as set forth in the references presented by the Examiner. Contrary to later definitions of "promoter" the definition of "promoter" considered to be accurate with respect to the state of the art by one of ordinary skill would have been found in Lewin or Watson, or Muller, not Old. The Old definition of "promoter' is not commensurate with the level of one of

ordinary skill in the art, clearly someone having a Ph.D., at the time the claimed invention was made. Contrary to Applicants' further arguments, Applicants' definition is not consistent with the descriptions and definitions of promoters supplied by the Examiner at the interview. The examiner's references clearly set forth the fact that the transcription initiation region is different from either promoter or enhancers. Further, Applicants have dismissed this point as "the examiner getting bogged down into the dissection of a promoter into its components; i.e., the RNA polymerase recognition site, the RNA polymerase binding site, and the transcription initiation site". However, this so-called "minor point" is the basis for Applicants feeling of entitlement to interference proceedings clearly set forth in the interview. 35 U.S.C. 112, first paragraph, requires an adequate written description of the invention sought to be patented and it is clear from the '069 application that the use of homologous recombination to integrate in heterologous promoters per se was never disclosed. In view of the fact that Applicants clearly set forth such ideas such as "enhancers", "wild-type promoters", "amplifiable genes", "signal leader sequence" and failed to clearly set forth the use of "promoters" alone, it is the examiner's position after considering all the evidence, that the preponderance of evidence indicates that the use of promoters per se was not included in the specification. Therefore, there is no

disclosure in the '069 specification for the use of heterologous promoters per se via homologous recombination.

Applicants have further argued, page 20, bottom paragraph, that the '069 application further includes a description of the use of targeted homologous recombination to further modify the target gene of interest in order to improve expression and production of the gene product. For example, the specification describes changing the signal leader sequence to promote secretion of the gene product and changing the 3' region, for example, the untranslated region, polyadenylation site, of the target gene. However, this argument is immaterial to the insertion of a heterologous promoter alone via homologous recombination. Further, it clearly provides evidence that each regulatory region of the gene necessary for expression was well known at the time the claimed invention was made.

Applicants have argued that the very title of the chapter in Lewin relied on by the Examiner: "Promoters: The Sites for Initiating Transcription" does not support the Examiner's strained interpretation. However, contrary to Applicants' arguments, the preponderance of evidence clearly indicates that the "promoter" was clearly distinguishable from other regulatory elements and sequences at the time the claimed invention was made and there is no support in the '069 specification for the use of heterologous promoters per se via homologous recombination.

Further, one of ordinary skill in the art of molecular biology would not rely on a chapter title to assign meaning. In view of the fact that many other regulatory elements were clearly set forth in the specification, and the use of heterologous promoters alone was not, the examiner concludes based on the evidence that the use of heterologous promoters per se in homologous recombination was not contemplated either in the specification or in the claims as originally filed.

Therefore, in view of the foregoing the claims of the 08/102,390 application are not entitled to the filing date of the parent application '069 with regard to the use of wild-type promoters alone in homologous recombination.

The rejection of claims 26, 27, 32-44, 46-66, 69, 70, 73-75,77-82, 84-88, 91, 92, 94-98, 100, 102-104 under 35 U.S.C. 112, second paragraph, regarding the phrase "modified endogenous gene" is withdrawn in view of the amendments to the claims. Further, the rejection of the claims for lack of enablement regarding the situation where the target gene is inaccessible for homologous recombination is withdrawn.

The rejection of claims 26, 28-31, 73 and 74 under 35 U.S.C. 103 as being obvious over Thomas is withdrawn in view of the amendments to the claims. Note that claim 26 previously claimed a modified endogenous gene and Applicants have amended the claim to include the limitation that the endogenous target gene coding

sequence is not disrupted. Therefore, Applicant's amendments to the claims have necessitated a new ground of rejection over new art and this Action will be made Final.

The rejection of claims 26, 28, 29, 30, 31, 73 and 74 under 35 U.S.C. 103 as being obvious over Thomas is withdrawn in view of the amendments to the claims.

The rejection of claims 27, 32-43 and 72 under 35 U.S.C. 103 as being unpatentable over Thomas as applied to claims 26,28-31, 73 and 74 above and further in view of Anderson and Song is withdrawn in view of the amendments to the claims.

The rejection of claims 48-61, 67-70 and 75-96 under 35 U.S.C. 103 as being unpatentable over thomas taken with Anderson and Song is withdrawn in view of the amendments to the claims.

The rejection of claims 44, 46, 47, 62 and 97-99 under 35 U.S.C. as being unpatentable over Liskay taken with Thomas is withdrawn in view of the amendments to the claims.

The rejection of claims 63-66 and 100-104 under 35 U.S.C. 103 as being unpatentable over Liskay taken with Thomas, Anderson and Song is withdrawn in view of the amendments to the claims.

In view of the amendments to the claims, the outstanding rejections have been withdrawn. Therefore, Applicant's arguments are moot. However, any arguments remaining pertinent are addressed below.

Claims 27, 32-44, 46-47, 49-61, 63-66, 73,75, 77-82, 84-88, 91, 98, 100, 102-104 are rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. The claims are vague and unclear since the condition of having an amplifiable gene integrated into the genome WITHIN or proximal to an endogenous target gene is inconsistent with the limitation that the endogenous target gene coding sequence not be disrupted. The amendments to the claims now present an inconsistency rendering the claim vague and unclear.

Claims 26, 38-41, 69 and 70 are rejected under 35 U.S.C. § 103 as being unpatentable over Smithies et al. and Nandi et al. taken with Thompson et al. Smithies discloses insertion of DNA sequences into the human chromosomal beta-globin locus by homologous recombination. Smithies discloses that the 5' end of the beta-globin gene was altered by using homologous recombination. Smithies discloses that the 5' part of the beta-globin gene includes the first exon and most of the second exon and the first intervening sequence but does not include the second intervening sequence. (Figure 1). Nandi discloses that the beta globin test gene was expressed under the control of the inserted regulatory sequences, see Figure 1, figure legend and figure 1b, and that the resident beta-globin was also expressed.

Nandi discloses that the expression of the endogenous target gene was controlled by the integrated regulatory elements on page 3848, column 2, top paragraph. Therefore, Smithies and Nandi disclose using homologous recombination to alter the 5' region of a naturally occurring beta-globin gene. Smithies and Nandi differ from the claims in that the references fail to disclose using homologous recombination to insert a nucleotide regulatory sequence per se. However, the secondary reference, Thompson, cures the deficiency.Thompson discloses the desirability of modifying the regulatory regions controlling gene expression. It would have been obvious to one of ordinary skill to modify the host cell of Smithies by altering the nucleotide regulatory sequences alone in order to control the expression of the endogenous target gene by the integrated regulatory sequence in view of the teachings of Thompson. Thompson discloses (page 313, column 1, first paragraph under "Introduction") that

"It would become possible to manipulate the expression of genes by targeting changes to their control sequences. This would be of value to the study of gene expression. It could also be of potential commercial value if used, for example, in liver-stock animals to increase output, or produce novel materials. In addition, genes could be inactivated to create animal models for human genetic diseases or to study the action of developmental genes, while the ability to correct mutant genes has implications for gene therapy. An advantage of being able to target modifications is that genes are manipulated in their natural chromosomal environment, whereas the use of conventional methods for introducing DNA sequences into the germ line (Jaenisch, 1988) allows no control over the chromosomal site of integration or the number of copies introduced".

Thus, Thompson clearly provides the motivation for targeting modifications to the regulatory regions of genes in order to manipulate the expression of the genes in their natural chromosomal environment. In view of the foregoing, Thompson provides the motivation to combine the references. The combination of references renders obvious the claimed mammalian host cell.

Regarding claim 26, Nandi discloses that the target gene is beta-globin and discloses that homologous recombination between the resident human beta-globin locus and the 5' sequences flanking the ^beta-test gene was detected (page 3846, column 1, last sentence). Nandi discloses that the beta globin test gene was expressed under the control of the inserted regulatory sequences, see Figure 1, figure legend and figure 1b, and that the resident beta-globin was also expressed. Nandi discloses that the expression of the endogenous target gene was controlled by the integrated regulatory elements on page 3848, column 2, top paragraph.

Regarding claims 38, 39, 40, 41, Smithies and Nandi disclose use of human EJ bladder carcinoma cells and therefore disclose use of primate cells (38), human cells (39), carcinoma cells (neoplastic cells) and somatic cells (bladder).

Regarding claims 69 and 70, Thompson clearly discloses the desirability of producing proteins via a method involving

homologous recombination by culturing the host cell and both Nandi and Smithies in fact showed expression of both the inserted gene and the resident gene.

Accordingly, the modification of the mammalian host cell of Smithies and Nandi by having a regulatory sequence different from the wild type integrated into the genome as suggested by Thompson in order to obtain a mammalian host cell having an endogenous target gene controlled by the integrated regulatory sequence was within the ordinary skill in the art at the time the claimed invention was made. From the teachings of the references, it is apparent that one of ordinary skill would have had a reasonable expectation of success in producing the claimed invention. Therefore, the invention as a whole is prima facie obvious, as evidenced by the references, especially in the absence of evidence to the contrary.

Claims 37 and 42 are rejected under 35 U.S.C. § 103 as being unpatentable over Smithies and Nandi taken with Thompson as applied to claims 26, 38-41, 69 and 70 above, and further in view of Palmer et al (PNAS 84: 1055, 1987, hereafter 'Palmer, 1987'). Palmer discloses methods of obtaining primary human diploid skin fibroblasts and methods of transfecting the cells. It would have been obvious to one of ordinary skill to substitute the skin fibroblasts of Palmer (1987) for the mammalian host cells of Smithies, Nandi and Thompson in order to obtain a host cell

having an endogenous target gene under the control of the integrated regulatory sequence. One of ordinary skill would have had a reasonable expectation of success in obtaining expression of the endogenous gene in view of the teachings of Smithies and Nandi that unexpressed genes could be targeted as successfully as expressed genes.

Accordingly, the modification of the mammalian host cell of Smithies, Nandi and Thompson by substituting human diploid skin fibroblasts as suggested by Palmer in order to obtain a mammalian host cell having an endogenous target gene controlled by the integrated regulatory sequence was within the ordinary skill in the art at the time the claimed invention was made. From the teachings of the references, it is apparent that one of ordinary skill would have had a reasonable expectation of success in producing the claimed invention. Therefore, the invention as a whole is prima facie obvious, as evidenced by the references, especially in the absence of evidence to the contrary.

Claims 33-36 are rejected under 35 U.S.C. § 103 as being unpatentable over Smithies, Nandi and Thompson as applied to claims 26, 38-41, 69 and 70 above, and further in view of Frohman et al. and Thomas et al. Claims 26, 38-41, 69 and 70 were rejected for reasons as stated above. Frohman discloses that homologous recombination has been exploited to deliberately alter endogenous genes by replacing them with "HR" constructs in which

the coding sequences has been interrupted. Therefore, Frohman discloses use of homologous recombination to introduce a mutation within the coding region of the endogenous gene. (page 145, column 1, second full paragraph). Thomas (Thomas and Capecchi, 1987, of record) used vectors containing a modified neomycin resistance gene inserted into the eighth exon the HPRT gene.

Regarding claims 33 and 34, the neo gene has the dual functional of disrupting the HPRT coding sequence and providing an additional selectable marker and that homologous recombination between the vector and the chromosomal HPRT gene resulted in the inactivation of the chromosomal gene. Thomas therefore discloses a host having a mutation within the amino acid coding region of the endogenous gene.

Regarding claims 35 and 36, Thomas discloses "The transfer of information by homologous recombination allows one to mutate or correct the desired chromosomal locus in a defined manner". Therefore, it would have been obvious to one of ordinary skill to modify the target gene in the 5' region, the 3' region or in any other desired site. One of ordinary skill would have had a reasonable expectation of success in obtaining such modifications since Thomas was able to show successful modification of the coding region and the technique would be the same with the only change occurring the nucleotide sequence to be targeted. Further Thompson clearly discloses the desirability of targeting

modifications to the promoter and 3' untranslated regions (page 319, column 2, last full paragraph).

Accordingly, the modification of the mammalian host cell of Smithies, Nandi and Thompson by altering coding and non-coding regions of the target gene as suggested by Frohman and Thomas in order to obtain a mammalian host cell having an endogenous target gene controlled by the integrated regulatory sequence was within the ordinary skill in the art at the time the claimed invention was made. From the teachings of the references, it is apparent that one of ordinary skill would have had a reasonable expectation of success in producing the claimed invention. Therefore, the invention as a whole is prima facie obvious, as evidenced by the references, especially in the absence of evidence to the contrary.

Claims 27, 32, 43, 91, 92 and 94 are rejected under 35 U.S.C. § 103 as being unpatentable over Smithies, Nandi and Thompson as applied to claims 26, 38-41, 69 and 70 above, and further in view of Anderson (of record) and Ringold (USPN 4,656,134). Claims 26, 38-41, 69 and 70 were rejected for reasons as stated above. Ringold discloses gene amplification in eukaryotic cells. Ringold further discloses that the DNA unit (column 2) will have the replication sequence which would be able to integrate into the chromosome either due to the presence of a translocatable sequence, such as an insertion sequence or

transposon, due to substantial homology with a sequence present in the chromosome and therefore clearly suggests integration by homologous recombination. Ringold further discloses and defines what constitutes the "amplifiable gene". Ringold discloses that various genes may be employed (column 2, lines 36-end) such as the gene expressing DHRF, and genes expressing metallothionein and others. Ringold discloses that by stressing the cells with agents such as methotrexate or metals in the case of the metallothioneine genes, that the cellular response will be amplification of the particular gene and flanking sequences, particularly a downstream flanking sequence. Ringold discloses that the DNA of interest may be any gene and most usually it will be desirable that the DNA sequence code for a polypeptide expression product, whose presence in the cell may be desirable to provide for a product. Ringold then discloses that the polypeptides may be enzymes hormones, lymphokines and other proteins clearly known in the art to be of therapeutic value. Ringold then clearly discloses that the target gene is also amplifiable. Anderson discloses the role of regulatory elements such as enhancers in gene therapy and further discloses that enhancers can be used to increase the properly regulated level of expression. Anderson further discloses the desirability of modifying germ cells or somatic cells to treat genetic disease. Anderson further teaches the use of amplifiable genes such the

metallothionein promoted gene on page 406,column 3, "Regulation by genomic control signals".

Regarding claims 91, 92 and 94, it would have been obvious to one of ordinary skill to integrate an amplifiable gene into the genome in order to increase the properly regulated level of expression in view of the teachings of Anderson that alteration of genomic regulatory signals is one method of increasing the expression of desirable genes and in view of the teachings of Ringold of the desirability of increasing the production of desired polypeptides having an obvious therapeutic value. Both Ringold and Anderson therefore provide the motivation to combine the references.

Accordingly, the modification of the mammalian host cell of Smithies, Nandi and Thompson by using homologous recombination to further integrate an amplifiable gene so as to also amplify the endogenous gene as suggested by Ringold and Anderson in order to obtain a mammalian host cell having an endogenous target gene controlled by the integrated regulatory sequence was within the ordinary skill in the art at the time the claimed invention was made. From the teachings of the references, it is apparent that one of ordinary skill would have had a reasonable expectation of success in producing the claimed invention. Therefore, the invention as a whole is prima facie obvious, as evidenced by the

references, especially in the absence of evidence to the contrary.

Claims 44, 46, 47 are rejected under 35 U.S.C. § 103 as being unpatentable over Smithies, Nandi and Thompson as applied to claims 26, 38-41, 69 and 70 above, and further in view of Nelson et al. Claims 26, 38-41, 69 and 70 were rejected for reasons as stated above. Nelson discloses transfer of chromosomes containing the gene of interest into a secondary host cell. Nelson further discloses transfer if several mouse chromosomes segments to both hamster and monkey cells. Nelson discloses that the donor cells were blocked in mitosis, the chromosomes removed and collected and then applied to CV-1 cells, CHTG49 cells or LA9 cells all of which are continuous cell lines (page 568). See figure 2 wherein the process of construction of chromosomes transferents is disclosed. See also page 570, column 1, bottom paragraph and top of next column. Nelson provides the motivation to combine the references on page 574, Discussion, wherein Nelson discloses that recipients of the chromosomes can be chosen from any cultured cell line that is able to take up DNA. Nelson further discloses (page 575) that the method can be applied to specific regions of the genome that do not currently contain a selectable marker. Nelson further discloses that this would allow detailed study of chromosome regions without the difficulties of isolating adjacent DNA from an entire genomic library.

Regarding claims 46 and 47, the secondary recipients are mammalian cells and Nelson discloses use of both monkey and hamster cells. The choice of cell line is immaterial, lacking evidence to the contrary, in view of the teachings of Nelson that any cell line is appropriate as long as the cells are capable of taking up DNA.

Accordingly, the modification of the mammalian host cell of Smithies, Nandi and Thompson by using a secondary expression host cell as suggested by Nelson in order to obtain a mammalian host cell having an endogenous target gene whose expression is controlled by the integrated regulatory element was within the ordinary skill in the art at the time the claimed invention was made. From the teachings of the references, it is apparent that one of ordinary skill would have had a reasonable expectation of success in producing the claimed invention. Therefore, the invention as a whole is prima facie obvious, as evidenced by the references, especially in the absence of evidence to the contrary.

Claims 48, 50, 52-55 and 79 are rejected under 35 U.S.C. § 103 as being unpatentable over Smithies et al. and Nandi et al. taken with Thompson et al. Smithies discloses a method for producing a mammalian host cell having a modified endogenous target gene using homologous recombination. Smithies discloses insertion of DNA sequences into the human chromosomal beta-globin

locus by homologous recombination. Smithies discloses that the 5' end of the beta-globin gene was altered by using homologous recombination. Smithies discloses that the 5' part of the beta-globin gene includes the first exon and most of the second exon and the first intervening sequence but does not include the second intervening sequence. (Figure 1). Nandi discloses that the beta globin test gene was expressed under the control of the inserted regulatory sequences, see Figure 1, figure legend and figure 1b, and that the resident beta-globin was also expressed. Nandi discloses that the expression of the endogenous target gene was controlled by the integrated regulatory elements on page 3848, column 2, top paragraph. Therefore, Smithies and Nandi disclose a method for producing a mammalian host cell having an endogenous target gene modified by using homologous recombination to alter the 5' region of a naturally occurring beta-globin gene. Smithies and Nandi differ from the claims in that the references fail to disclose using homologous recombination to insert a nucleotide regulatory sequence per se. However, the secondary reference, Thompson, cures the deficiency. Thompson discloses the desirability of modifying the regulatory regions controlling gene expression. It would have been obvious to one of ordinary skill to modify the method of Smithies and Nandi by altering the nucleotide regulatory sequences alone in order to control the expression of the endogenous target gene by the integrated

regulatory sequence in view of the teachings of Thompson. Thompson discloses (page 313, column 1, first paragraph under "Introduction") that

"It would become possible to manipulate the expression of genes by targeting changes to their control sequences. This would be of value to the study of gene expression. It could also be of potential commercial value if used, for example, in liver-stock animals to increase output, or produce novel materials. In addition, genes could be inactivated to create animal models for human genetic diseases or to study the action of developmental genes, while the ability to correct mutant genes has implications for gene therapy. An advantage of being able to target modifications is that genes are manipulated in their natural chromosomal environment, whereas the use of conventional methods for introducing DNA sequences into the germ line (Jaenisch, 1988) allows no control over the chromosomal site of integration or the number of copies introduced".

Thus, Thompson clearly provides the motivation for targeting modifications to the regulatory regions of genes in order to manipulate the expression of the genes in their natural chromosomal environment. In view of the foregoing, Thompson provides the motivation to combine the references. The combination of references renders obvious the claimed mammalian host cell.

Regarding claim 48, Nandi discloses that the target gene is beta-globin and discloses that homologous recombination between the resident human beta-globin locus and the 5' sequences flanking the ^beta-test gene was detected (page 3846, column 1, last sentence). Nandi discloses that the beta globin test gene was expressed under the control of the inserted regulatory

sequences, see Figure 1, figure legend and figure 1b, and that the resident beta-globin was also expressed. Nandi discloses that the expression of the endogenous target gene was controlled by the integrated regulatory elements on page 3848, column 2, top paragraph.

Regarding claims 50 and 79, Smithies discloses use of the Neo gene as a selectable marker, which would be selectable with G418.

Regarding claims 52-55, Smithies and Nandi disclose use of human EJ bladder carcinoma cells and therefore disclose use of primate cells (52), human cells (53), carcinoma cells (neoplastic cells, 54) and somatic cells (bladder, 55).

Accordingly, the modification of the method of Smithies and Nandi by having a regulatory sequence different from the wild type integrated into the genome as suggested by Thompson in order to obtain a method for producing a mammalian host cell having an endogenous target gene controlled by the integrated regulatory sequence was within the ordinary skill in the art at the time the claimed invention was made. From the teachings of the references, it is apparent that one of ordinary skill would have had a reasonable expectation of success in producing the claimed invention. Therefore, the invention as a whole is prima facie obvious, as evidenced by the references, especially in the absence of evidence to the contrary.

Claims 51 and 56 are rejected under 35 U.S.C. § 103 as being unpatentable over Smithies and Nandi taken with Thompson as applied to claims 48, 50, 52-55 and 79 above, and further in view of Palmer et al (PNAS 84: 1055, 1987, hereafter 'Palmer, 1987'). Palmer discloses methods of obtaining primary human diploid skin fibroblasts and methods of transfecting the cells. It would have been obvious to one of ordinary skill to substitute the skin fibroblasts of Palmer (1987) for the mammalian host cells of Smithies, Nandi and Thompson in order to obtain a host cell having an endogenous target gene under the control of the integrated regulatory sequence. One of ordinary skill would have had a reasonable expectation of success in obtaining expression of the endogenous gene in view of the teachings of Smithies and Nandi that unexpressed genes could be targeted as successfully as expressed genes.

Accordingly, the modification of the method of Smithies, Nandi and Thompson by substituting human diploid skin fibroblasts as suggested by Palmer in order to obtain a method for producing a mammalian host cell having an endogenous target gene controlled by the integrated regulatory sequence was within the ordinary skill in the art at the time the claimed invention was made. From the teachings of the references, it is apparent that one of ordinary skill would have had a reasonable expectation of success in producing the claimed invention. Therefore, the invention as a

whole is prima facie obvious, as evidenced by the references, especially in the absence of evidence to the contrary.

Claims 58-61 are rejected under 35 U.S.C. § 103 as being unpatentable over Smithies, Nandi and Thompson as applied to claims 48, 50, 52-55 and 79 above, and further in view of Frohman et al. and Thomas et al. Frohman discloses that homologous recombination has been exploited to deliberately alter endogenous genes by replacing them with "HR" constructs in which the coding sequences has been interrupted. Therefore, Frohman discloses use of homologous recombination to introduce a mutation within the coding region of the endogenous gene. (page 145, column 1, second full paragraph). Thomas (Thomas and Capecchi, 1987, of record) used vectors containing a modified neomycin resistance gene inserted into the eighth exon the HPRT gene.

Regarding claims 58 and 59, the neo gene has the dual functional role of disrupting the HPRT coding sequence and providing an additional selectable marker and that homologous recombination between the vector and the chromosomal HPRT gene resulted in the inactivation of the chromosomal gene. Thomas therefore discloses a host having a mutation within the amino acid coding region of the endogenous gene.

Regarding claims 60 and 61, Thomas discloses "The transfer of information by homologous recombination allows one to mutate or correct the desired chromosomal locus in a defined manner".

Therefore, it would have been obvious to one of ordinary skill to modify the target gene in the 5' region, the 3' region or in any other desired site. One of ordinary skill would have had a reasonable expectation of success in obtaining such modifications since Thomas was able to show successful modification of the coding region and the technique would be the same with the only change occurring the nucleotide sequence to be targeted. Further Thompson clearly discloses the desirability of targeting modifications to the promoter and 3' untranslated regions (page 319, column 2, last full paragraph).

Accordingly, the modification of the method of Smithies, Nandi and Thompson by altering coding and non-coding regions of the target gene as suggested by Frohman and Thomas in order to obtain a method for producing a mammalian host cell having an endogenous target gene controlled by the integrated regulatory sequence was within the ordinary skill in the art at the time the claimed invention was made. From the teachings of the references, it is apparent that one of ordinary skill would have had a reasonable expectation of success in producing the claimed invention. Therefore, the invention as a whole is prima facie obvious, as evidenced by the references, especially in the absence of evidence to the contrary.

Claims 49, 57, 75, 80 and 81 are rejected under 35 U.S.C. § 103 as being unpatentable over Smithies, Nandi and Thompson as

applied to claims 48, 50, 52-55 and 79 above, and further in view of Anderson (of record) and Ringold (USPN 4,656,134). Ringold discloses gene amplification in eukaryotic cells. Ringold further discloses that the DNA unit (column 2) will have the replication sequence which would be able to integrate into the chromosome either due to the presence of a translocatable sequence, such as an insertion sequence or transposon, due to substantial homology with a sequence present in the chromosome and therefore clearly suggests integration by homologous recombination. Ringold further discloses and defines what constitutes the "amplifiable gene". Ringold discloses that various genes may be employed (column 2, lines 36-end) such as the gene expressing DHRF, and genes expressing metallothionein and others. Ringold discloses that by stressing the cells with agents such as methotrexate or metals in the case of the metallothioneine genes, that the cellular response will be amplification of the particular gene and flanking sequences, particularly a downstream flanking sequence. Ringold discloses that the DNA of interest may be any gene and most usually it will be desirable that the DNA sequence code for a polypeptide expression product, whose presence in the cell may be desirable to provide for a product. Ringold then discloses that the polypeptides may be enzymes hormones, lymphokines and other proteins clearly known in the art to be of therapeutic value. Ringold then clearly discloses that the target gene is

also amplifiable. Anderson discloses the role of regulatory elements such as enhancers in gene therapy and further discloses that enhancers can be used to increase the properly regulated level of expression. Anderson further discloses the desirability of modifying germ cells or somatic cells to treat genetic disease. Anderson further teaches the use of amplifiable genes such the metallothionein promoted gene on page 406, column 3, "Regulation by genomic control signals". It would have been obvious to one of ordinary skill to integrate an amplifiable gene into the genome in order to increase the properly regulated level of expression in view of the teachings of Anderson that alteration of genomic regulatory signals is one method of increasing the expression of desirable genes and in view of the teachings of Ringold of the desirability of increasing the production of desired polypeptides having an obvious therapeutic value. Both Ringold and Anderson therefore provide the motivation to combine the references.

Regarding claims 80 and 81, the use of any selectable marker system is routine to one of ordinary skill and the use of the tk marker as a negative selectable marker gene is merely a choice amongst equivalents. Note that the specification discloses use of multiple selection systems.

Accordingly, the modification of the method of Smithies, Nandi and Thompson by using homologous recombination to further

integrate an amplifiable gene so as to also amplify the endogenous gene as suggested by Ringold and Anderson in order to obtain a method for producing a mammalian host cell having an endogenous target gene controlled by the integrated regulatory sequence was within the ordinary skill in the art at the time the claimed invention was made. From the teachings of the references, it is apparent that one of ordinary skill would have had a reasonable expectation of success in producing the claimed invention. Therefore, the invention as a whole is prima facie obvious, as evidenced by the references, especially in the absence of evidence to the contrary.

Claims 62, 64-66, 86, 97 and 102 are rejected under 35 U.S.C. § 103 as being unpatentable over Nelson taken with Smithies, Nandi and Thompson. Nelson discloses a method for producing a secondary expression host cell. Nelson discloses transfer of chromosomes containing the gene of interest into a secondary host cell. Nelson further discloses transfer of several mouse chromosome segments to both hamster and monkey cells. Nelson discloses that the donor cells were blocked in mitosis, the chromosomes removed and collected and then applied to CV-1 cells, CHTG49 cells or LA9 cells all of which are continuous cell lines (page 568). See figure 2 wherein the process of construction of chromosome transferents is disclosed. See also page 570, column 1, bottom paragraph and top of next column.

Nelson provides the motivation to combine the references on page 574, Discussion, wherein Nelson discloses that recipients of the chromosomes can be chosen from any cultured cell line that is able to take up DNA. Nelson further discloses (page 575) that the method can be applied to specific regions of the genome that do not currently contain a selectable marker. Nelson further discloses that this method of chromosome transfer would allow detailed study of chromosome regions without the difficulties of isolating adjacent DNA from an entire genomic library. Nelson differs from the claims in that the reference fails to disclose a mammalian host cell having an endogenous gene modified by integration of regulatory sequences different from the wild type. However, the secondary references, Smithies, Nandi and Thompson, taken together, cure the deficiency. Smithies discloses insertion of DNA sequences into the human chromosomal beta-globin locus by homologous recombination. Smithies discloses that the 5' end of the beta-globin gene was altered by using homologous recombination. Smithies discloses that the 5' part of the beta-globin gene includes the first exon and most of the second exon and the first intervening sequence but does not include the second intervening sequence. (Figure 1). Nandi discloses that the beta globin test gene was expressed under the control of the inserted regulatory sequences, see Figure 1, figure legend and figure 1b, and that the resident beta-globin was also expressed.

Nandi discloses that the expression of the endogenous target gene was controlled by the integrated regulatory elements on page 3848, column 2, top paragraph. Therefore, Smithies and Nandi disclose using homologous recombination to alter the 5' region of a naturally occurring beta-globin gene. It would have been obvious to one of ordinary skill to modify the host cell of Smithies and Nandi by altering the nucleotide regulatory sequences alone in order to control the expression of the endogenous target gene by the integrated regulatory sequence in view of the teachings of Thompson. Thompson discloses (page 313, column 1, first paragraph under "Introduction") that

> "It would become possible to manipulate the expression of genes by targeting changes to their control sequences. This would be of value to the study of gene expression. It could also be of potential commercial value if used, for example, in liver-stock animals to increase output, or produce novel materials. In addition, genes could be inactivated to create animal models for human genetic diseases or to study the action of developmental genes, while the ability to correct mutant genes has implications for gene therapy. An advantage of being able to target modifications is that genes are manipulated in their natural chromosomal environment, whereas the use of conventional methods for introducing DNA sequences into the germ line (Jaenisch, 1988) allows no control over the chromosomal site of integration or the number of copies introduced".

Thus, Thompson clearly provides the motivation for targeting modifications to the regulatory regions of genes in order to manipulate the expression of the genes in their natural chromosomal environment. In view of the foregoing, Thompson provides the motivation to combine the references.

Regarding claims 64 and 86, Nelson discloses use of the Neo gene, a positive selection marker and selection with G418.

Regarding claims 65 and 66, the secondary recipients are mammalian cells and Nelson discloses use of both monkey and hamster cells. The choice of cell line is immaterial, lacking evidence to the contrary, in view of the teachings of Nelson that any cell line is appropriate as long as the cells are capable of taking up DNA.

Regarding claims 97 and 102, it would have been obvious to one of ordinary skill to use the secondary expression host cells to produce the protein in view of the teachings of Thompson disclosing the desirability of producing therapeutic products by manipulating the regulatory regions of genes.

Accordingly, the modification of the method of Nelson by using the DNA derived from the mammalian host cell of Smithies, Nandi and Thompson in order to obtain a method for producing a secondary expression host cell was within the ordinary skill in the art at the time the claimed invention was made. From the teachings of the references, it is apparent that one of ordinary skill would have had a reasonable expectation of success in producing the claimed invention. Therefore, the invention as a whole is prima facie obvious, as evidenced by the references, especially in the absence of evidence to the contrary.

Claims 63, 82, 87, 88, 98 and 100 are rejected under 35 U.S.C. § 103 as being unpatentable over Nelson taken with Smithies, Nandi and Thompson as applied to claims 62, 64-66, 86, 97 and 102 above, and further in view of Ringold and Anderson. Claims 62, 64-66, 86, 97 and 102 were rejected for reasons as stated above. Ringold discloses gene amplification in eukaryotic cells. Ringold further discloses that the DNA unit (column 2) will have the replication sequence which would be able to integrate into the chromosome either due to the presence of a translocatable sequence, such as an insertion sequence or transposon, due to substantial homology with a sequence present in the chromosome and therefore clearly suggests integration by homologous recombination. Ringold further discloses and defines what constitutes the "amplifiable gene". Ringold discloses that various genes may be employed (column 2, lines 36-end) such as the gene expressing DHRF, and genes expressing metallothionein and others. Ringold discloses that by stressing the cells with agents such as methotrexate or metals in the case of the metallothioneine genes, that the cellular response will be amplification of the particular gene and flanking sequences, particularly a downstream flanking sequence. Ringold discloses that the DNA of interest may be any gene and most usually it will be desirable that the DNA sequence code for a polypeptide expression product, whose presence in the cell may be desirable

to provide for a product. Ringold then discloses that the polypeptides may be enzymes hormones, lymphokines and other proteins clearly known in the art to be of therapeutic value. Ringold then clearly discloses that the target gene is also amplifiable. Anderson discloses the role of regulatory elements such as enhancers in gene therapy and further discloses that enhancers can be used to increase the properly regulated level of expression. Anderson further discloses the desirability of modifying germ cells or somatic cells to treat genetic disease. Anderson further teaches the use of amplifiable genes such the metallothionein promoted gene on page 406,column 3, "Regulation by genomic control signals". It would have been obvious to one of ordinary skill to integrate an amplifiable gene into the genome in order to increase the properly regulated level of expression in view of the teachings of Anderson that alteration of genomic regulatory signals is one method of increasing the expression of desirable genes and in view of the teachings of Ringold of the desirability of increasing the production of desired polypeptides having an obvious therapeutic value. Both Ringold and Anderson therefore provide the motivation to combine the references.

Regarding claims 87 and 88, the use of any selectable marker is old and well known in the art and merely a choice amongst equivalents.

Accordingly, the modification of the method of Nelson, Smithies, Nandi and Thompson by using homologous recombination to further integrate an amplifiable gene so as to also amplify the endogenous gene as suggested by Ringold and Anderson in order to obtain a method for producing a secondary expression host cell having an endogenous target gene controlled by the integrated regulatory sequence which was obtained from a primary cell was within the ordinary skill in the art at the time the claimed invention was made. From the teachings of the references, it is apparent that one of ordinary skill would have had a reasonable expectation of success in producing the claimed invention. Therefore, the invention as a whole is prima facie obvious, as evidenced by the references, especially in the absence of evidence to the contrary.

Claims 73, 74, 77, 78, 84, 85, 95, 96, 103 and 104 are rejected under 35 U.S.C. § 103 as being unpatentable over Smithies, Nandi, Thompson, or Nelson, Smithies, Nandi and Thompson or Smithies, Nandi, Thompson, Nelson, Ringold and Anderson as applied to claims 26, 27, 48, 7562, 82, 69, 91, 97, 98 above, and further in view of Foecking et al. and Boshart et al. Claims 26, 27, 48, 75, 62, 82, 69, 91, 97, 98 were rejected for reasons as stated above. Foecking and Boshart disclose the promoter/enhancer unit from CMV. Foecking discloses on page 101 that the CMV promoter has been reported to be a very strong

enhancer with little species or tissue specificity and that the level of expression of a foreign gene is determined by the strength of both the promoter and enhancer which drive its transcription. It would have been obvious to one of ordinary skill to substitute the CMV promoter of Foecking and Boshart for any promoter used by Nandi, Smithies, Ringold, Anderson and others in view of the teachings of Thompson to manipulate the expression of genes by targeting changes to the control regions of genes using homologous recombination. In view of the teachings of Foecking and Boshart, one of ordinary skill would have had a reasonable expectation of success in expressing genes using the CMV promoter/enhancer since the promoter/enhancer lacks both species and tissue specificity.

Accordingly, the modification of the method or host cells of Smithies, Nandi and Thompson or Nelson, Smithies, Nandi, and Thompson,or Smithies, Nandi, Thompson, Nelson, Ringold and Anderson by using homologous recombination to integrate the CMV promoter as suggested by Boshart and Foecking in order to obtain a host cell having an endogenous target gene controlled by the integrated regulatory sequence was within the ordinary skill in the art at the time the claimed invention was made. From the teachings of the references, it is apparent that one of ordinary skill would have had a reasonable expectation of success in producing the claimed invention. Therefore, the invention as a

whole is prima facie obvious, as evidenced by the references, especially in the absence of evidence to the contrary.

Applicant's arguments are moot in view of the new grounds of rejection necessitated by the amendments to the claims. However, the following comments regarding the art remain pertinent.

Applicants have argued on page 22 of the instant amendment that homologous recombination can be accomplished in a different host cell when problems are encountered in one particular type. The examiner agrees and points out that Applicant's arguments are commensurate with the new ground of rejection wherein it is stated that one of ordinary skill would have had a reasonable expectation of success in substituting the diploid fibroblast cell of Palmer for the human EJ carcinoma cell of Smithies and Nandi and obtaining a cell having an endogenous target gene comprising a nucleotide regulatory sequence different from the wild-type. Note that the claims do not claim large scale protein production.

Regarding the art rejections under 35 USC 103, Applicants have argued that none of the cited art suggests the use of targeted homologous recombination to activate gene expression. However, none of the claims claim activating endogenous gene expression.

Applicants have argued that Dr. Liskay has read the references and concluded that the cited art does not suggest the

unique combination of elements as utilized in the invention. However, the conclusion of Dr. Liskay on this issue is one of opinion only and not given probative weight.

Applicants have argued that Thomas is an inappropriate reference for reasons contained on page 27 and 28 of the instant amendment. However, Applicants' amendments to the claims have mooted the rejection and the arguments are not addressed except to note that the claims do not exclude ES cells. In view of the new grounds of rejection, the statement by Dr. Liskay in the declaration are no longer pertinent.

Applicants have argued that Anderson actually teaches away from the use of homologous recombination in mammalian cells. However, Anderson was cited to teach the suggestion of controlling protein expression by altering the regulatory regions of the genes and remains so cited. The issue of in vitro vs in vivo is not pertinent here since the claims are all drawn to in vitro manipulation.

Applicants have argued that Thompson is an inappropriate reference since Thompson is directed to a correction of a deletion mutation in a structural gene of ES cells. However, Thompson clearly discloses the desirability of manipulating the expression of genes by targeting changes to their control sequences and was cited to teach such. The fact that Thompson used ES cells is immaterial since the claims do not exclude ES

cells. Further, the opinion of Dr. Liskay as to what Thompson means is immaterial since it is opinion only.

Applicants have argued that the Examiner has used hindsight and that hindsight is being used is supported by Dr. Liskay's analysis. However, contrary to such opinions which have no probative weight, the prior art clearly teaches the use of homologous recombination to integrate DNA of any type into the genome, regardless of cell types. Once the mechanism of homologous recombination was shown to successfully integrate at all different sections of a gene, it would have been obvious to one of ordinary skill to alter the regulatory regions of genes in order to enhance the expression of the desired gene. Thompson clearly disclosed the suggestion for doing so. Lastly, the teachings of Milton are not the basis of patent law and the opinion of Dr. Liskay after learning of the invention in irrelevant because Thompson published the suggestion to target modifications to the control regions of genes in order to manipulate the expression of genes in their natural chromosomal environment.

No claim is allowed.

Applicant's amendment necessitated the new grounds of rejection. Accordingly, **THIS ACTION IS MADE FINAL.** See M.P.E.P. § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 C.F.R. § 1.136(a).

A SHORTENED STATUTORY PERIOD FOR RESPONSE TO THIS FINAL ACTION IS SET TO EXPIRE THREE MONTHS FROM THE DATE OF THIS

ACTION. IN THE EVENT A FIRST RESPONSE IS FILED WITHIN TWO MONTHS OF THE MAILING DATE OF THIS FINAL ACTION AND THE ADVISORY ACTION IS NOT MAILED UNTIL AFTER THE END OF THE THREE-MONTH SHORTENED STATUTORY PERIOD, THEN THE SHORTENED STATUTORY PERIOD WILL EXPIRE ON THE DATE THE ADVISORY ACTION IS MAILED, AND ANY EXTENSION FEE PURSUANT TO 37 C.F.R. § 1.136(a) WILL BE CALCULATED FROM THE MAILING DATE OF THE ADVISORY ACTION. IN NO EVENT WILL THE STATUTORY PERIOD FOR RESPONSE EXPIRE LATER THAN SIX MONTHS FROM THE DATE OF THIS FINAL ACTION.

Papers related to this application may be submitted to Group 1800 by facsimile transmission. Papers should be faxed to Group 1800 via the PTO FAX center located in Crystal Mall 1. The faxing of such papers must conform with the notice published in the Official Gazette, 1096 OG (30 November 15, 1989). The CM1 Fax Center number is (703) 308-4227.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Examiner Suzanne Ziska, Ph.D., whose telephone number is (703)308-1217.

If the Examiner cannot be reached, any inquiries concerning this communication or earlier communications from the Examiner should be directed to the Examiner's supervisor, Ms.Jacqueline Stone, whose telephone number is (703) 308-3153.

SUZANNE E. ZISKA
PRIMARY EXAMINER
GROUP 1800